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IN RE APPLICATION OF:
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FOR: IMMUNE ACTIVATOR

CERTIFICATE OF TRANSLATION

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Sir:

I, Toshio Miyake, a translator, with a Post Office Address of DaVinci Bosei 7F, 3-20-12 Shin-Yokohama, Kohoku-ku, Yokohama, Kanagawa-ken, Japan

hereby states:

(1) that I am fluent in both the Japanese and English languages;

(2) that I translated the attached document identified as corresponding to JP 2001-132513 filed in Japan on April 27, 2001, and bearing the Reference Number P6972AJ, from Japanese to English;

(3) that the attached English translation is a true and correct translation of 2001-132513, to the best of my knowledge and belief; and

(4) that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true and further, that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 USC 1001, and that such false statements may jeopardize the validity of the application or any patent issuing thereon.

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[Title of the Invention] IMMUNE ACTIVATOR

[Claims]

[Claim 1]

Superfine particles of a mushroom extract characterized by superfine pulverization of an extract of a mushroom.

[Claim 2]

The superfine particles according to claim 1, wherein the extract of a mushroom is a water extract.

[Claim 3]

The superfine particles according to claim 1 or 2, wherein an average particle diameter of the superfine particles, as determined in the form of a dispersion in water, is 10 μm or less.

[Claim 4]

The superfine particles according to claim 1, which comprise particles having an average particle diameter of 10 μm or less obtainable by mixing an emulsifier with an aqueous solution containing an extract of a mushroom obtained after separation of insoluble matters from a water or a hot-water extract of a mushroom.

[Claim 5]

The superfine particles according to claim 4, wherein the aqueous solution containing an extract of a mushroom is an aqueous solution containing aggregates obtained by separation of insoluble matters from a water or a hot-water extract of a mushroom and then concentrating and/or cooling the extract.

[Claim 6]

The superfine particles according to claim 4, wherein the aqueous solution containing an extract of a mushroom is a homogeneous aqueous solution, and, upon being concentrated and/or cooled, gives aggregates.

[Claim 7]

The superfine particles according to claim 3, wherein the average particle diameter is 1 μm or less.

[Claim 8]

The superfine particles according to claim 3, wherein the average particle diameter is 0.01 to 1 μm.

[Claim 9]

The superfine particles according to claim 1 or 2, which are in a state treated or dispersed with an emulsifier.

[Claim 10]

The superfine particles according to any one of claims 1 to 9, which are in the form of micelles.

[Claim 11]

The superfine particles according to any one of claims 1 to 10, which are in the form of liposomes.

[Claim 12]

An immune activator or an immune regulator characterized by comprising the superfine particles described in any one of claims 1 to 11.

[Claim 13]

An antitumor agent, an anti-infective agent, an antiviral agent, an anti-autoimmune disease agent, an anti-diabetes agent, or an anti-allergy agent, characterized by comprising the superfine particles described in any one of claims 1 to 11.

[Claim 14]

A pharmaceutical composition comprising the superfine particles described in any one of claims 1 to 11 and optionally a pharmaceutically acceptable carrier or excipient (bulking filler).

[Claim 15]

Food and drink comprising the superfine particles described in any one of claims 1 to 11.

[Claim 16]

The food and drink according to claim 15, which comprise the superfine particles described in any one of claims 1 to 11 in an amount of 0.01 to 20% by weight.

[Claim 17]

The food and drink according to claim 15 or 16, which is used in patients with a disease selected from a cancer disease, a microbial infectious disease, a viral infectious disease, an autoimmune diseases, diabetes, and an allergy.

[Claim 18]

A process for producing superfine particles of a mushroom extract characterized by subjecting a mushroom to a step of extraction by water, and then subjecting the water extract to a step of superfine pulverization.

[Claim 19]

A process for producing superfine particles containing particles having an average particle diameter of 10 μm or less, characterized by mixing an emulsifier with an aqueous solution containing an extract of a mushroom obtained after separation of insoluble matters from a water or a hot-water extract of a mushroom.

[Detailed Description of the Invention]

[0001]

[Field of the Invention]

The present invention relates to novel superfine particles of a mushroom extract (preferably, superfine particles of a component obtained by extraction from a mushroom with water), an immune activator and/or an immune regulator comprising the superfine particles or the composition as an active ingredient (immune activator/immune regulator), a pharmaceutical composition (particularly, pharmaceutical preparations for diseases starting (occurring) due to abnormalities in immune functions, such as an antitumor agent, an anti-infective agent, an antiviral agent, an anti-autoimmune disease agent, an anti-diabetes agent and an anti-allergy agent, as well as food and drink (health foods, functional foods etc.), and a process for producing the superfine particles usable as an active ingredient for these diseases.

[0002]

The immune activator/immune regulator of the present invention is used in various forms such as a pharmaceutical preparation (pharmaceutical composition), food and drink (health foods, functional

foods etc.) etc., and is useful for treatment, amelioration, and prevention from progression, of diseases particularly by activating or regulating immune functions, for prophylaxis of other diseases occurring due to abnormalities in immune functions in (for) patients and the like, and for prophylaxis of various diseases accompanying abnormalities in immune functions for healthy persons by activating or regulating immune functions, and for amelioration of light diseases by improving immune functions therefor, and the like.

[0003]

[Background of the Invention]

Mushrooms or components thereof contain various pharmaceutically efficacious components, and thus health foods containing processed powder of a certain mushroom or a hot-water extract thereof are known. In the conventionally known products, however, their various components are not sufficiently utilized.

[0004]

Accordingly, it is expected that various components of mushrooms are utilized effectively much more in the form of pharmaceutical preparations, health foods or functional foods in comparison to the conventional products, in order to maintain and improve the health of animals, particularly humans in daily life, or used in pharmaceutical preparations to treat or ameliorate diseases.

[0005]

[Problem to be Solved by the Invention]

The object of the present invention is to provide food and drink

(health foods, functional foods etc.) or a pharmaceutical preparation (pharmaceutical composition), which can be prepared by easy preparative means and effectively utilize various components particularly pharmaceutically efficacious components in mushrooms.

[0006]

[Means to Solve the Problem]

To solve the problem described above, the present inventors made extensive study on utilization of various components in conventional products, and as a result, they found that the various components were not sufficiently absorbed into animal bodies, and were thus not so utilized as to be expected in the bodies. As a result of further investigation, the inventors found that when an extract of a mushroom with water, preferably an extract thereof with hot water, were further finely pulverized to prepare superfine particles and dispersed, for example in water such that an average particle diameter of the superfine particles was 10 μm or less, more preferably 1 μm or less, still more preferably 0.01 to 1 μm in a micellar state, incorporation thereof through mucosa was significantly improved, and as a result, immune functions could be activated or regulated.

[0007]

On the basis of the findings described above, the present invention was arrived at. Particularly, mucosal immunity is stimulated and activated by incorporation through mucosa (particularly the small intestine) into the body (leading to activation of systemic immunity), and as a result, an antitumor effect or a therapeutic treating and/or

ameliorating effect on infectious diseases with viruses, such as AIDS, and bacteria or the like, can be expected. Accordingly, the superfine particles can be used as an immune activator/immune regulator (which is an immune activator and/or an immune regulator), and use thereof in the form of pharmaceutical composition(s) or food(s) and drink(s) (which is/are foods(s) and/or drink(s)) (health foods etc.) can be expected.

[0008]

That is, one aspect of the present invention lies in superfine particles of a mushroom extract (an extract of a mushroom) characterized in that an extract of a mushroom is converted into superfine particles.

[0009]

The extract of a mushroom is preferably an extract thereof with water (a water extract of a mushroom) (including extracts with water, hot water, a water-containing solution, etc.) in order to obtain a larger amount of the active (effective) ingredient in the present invention. The extract of a mushroom with water (the water extract of a mushroom) may be component(s) extracted from a mushroom with water or a material containing the component(s), and the extract includes, for example, a homogeneous aqueous solution obtained in a step of extraction of a mushroom with water after removing insoluble matters, which can not be extracted with water, by filtration or the like, components contained therein (in the from of solid(s) or an aqueous solution, etc.), and a dispersion containing, in the aqueous solution, a

part of the water-extracted component(s) in the form of dispersed fine particles (e.g. precipitates of aggregates each having a particle diameter of 100 μm or more, etc.). Thereafter, such a water extract of a mushroom can be subjected to the step of superfine pulverization in the present invention for superfine particles preparation.

[0010]

The superfine particles of a mushroom extract have an average particle diameter of preferably 10 μm or less, more preferably 1 μm or less, still more preferably 0.01 to 1 μm or so as determined after being dispersed in water. This average particle diameter can be determined easily with a particle size distribution meter.

[0011]

The superfine particles can be obtained preferably by separating insoluble matters from an extract (solution) of a water or hot-water mushroom (through filtration or the like) to give an aqueous solution containing a mushroom extract, mixing an emulsifier therewith preferably under stirring. The superfine particles containing particles having an average particle diameter of 10 μm or less can be obtained in this manner.

[0012]

In this case, it is possible to use not only an aqueous solution containing aggregates obtained by separating insoluble matters from an extract of a water or hot-water mushroom as the aqueous mushroom extract-containing solution (the aqueous solution containing a mushroom extract) and then concentrating and/or cooling the extract,

but also a homogeneous aqueous solution which will, upon being concentrated and/or cooled, give the aggregates.

[0013]

For use as an immune activator or an immune regulator, particularly, the superfine particles can be used in the form of an aqueous solution or emulsion in which the extract of a mushroom is finely (mushroom extract treated with an emulsifier as described above), desirably in the form of micelles or liposomes. Among these, the mushroom extract treated with an emulsifier can be conveniently used. Thus, the superfine particles in such various forms also fall under the scope of the superfine particles of a mushroom extract (an extract of a mushroom) of the present invention.

[0014]

The extract of a mushroom with hot water, is preferable from the viewpoint of efficient extraction of the active (effective) ingredient with hot water from a mushroom after milling.

[0015]

The superfine particles of a mushroom extract can be absorbed or incorporated (ingested) through mucosa in small intestines of animals particularly humans, thus exhibiting an immune activating effect or an immune regulating effect.

[0016]

Another aspect of the present invention lies in an immune activator and/or an immune regulator characterized by comprising any superfine particles described above (immune activator/immune

regulator).

[0017]

A still other aspect of the present invention lies in a pharmaceutical composition characterized by comprising any superfine particles described above (agent (medicine)). The pharmaceutical composition may contain a pharmaceutically acceptable carrier, excipient (bulk filler) (or diluent) etc.

[0018]

The immune (immunity) activator/immune regulator can also be used in the form of food and drink (food and/or drink).

[0019]

Examples of the above medicine include an antitumor agent, an anti-infective agent, an antiviral agent, an anti-autoimmune disease agent, an anti-diabetes agent and an anti-allergy agent.

[0020]

Another aspect of the present invention lies in food and drink (food and/or drink) characterized by comprising any superfine particles described above. The content of the superfine particles is not limited, and the content of the superfine particles in food and drink such as health foods is preferably 0.01 to 20% by weight.

[0021]

The food and drink of the present invention can be used as health foods, functional foods, health drinks, functional drinks etc. The food and drink are particularly suitable for patients with diseases such as cancers, microbial infectious diseases, viral infectious diseases,

autoimmune diseases, diabetes, and allergy.

[0022]

Another aspect of the present invention lies in a process for producing the superfine particles of a mushroom extract characterized in that a mushroom is subjected to a step of extraction with water, and then the resulting aqueous extract is subjected to a step of superfine pulverization. In this case, the extract preferably having an immune activating activity and/or an immune regulating activity can be obtained.

[0023]

In particular, the present invention also lies in a process for producing the superfine particles containing particles having an average particle diameter of 10 μm or less characterized by separating insoluble matters from a water or hot-water extract of a mushroom to give an aqueous mushroom extract-containing solution (an aqueous solution containing an extract of a mushroom) and then mixing an emulsifier therewith preferably under stirring. In this case, it is possible to use not only an aqueous solution containing aggregates obtained by separating insoluble matters from a water or hot-water extract of a mushroom as the aqueous mushroom extract-containing solution (aqueous solution containing the extract thereof) and then concentrating and/or cooling the extract, but also a homogeneous aqueous solution as it is which will, upon being concentrated and/or cooled, give the aggregates, as described above.

[0024]

The average particle diameter refers to the average particle diameter of the particles measured (determined) in the form of a dispersion in water as described above.

[0025]

[Embodiments of the Invention]

Hereinafter, the mode for carrying out the invention is described in more detail.

[0026]

(Superfine particles of a mushroom extract (an extract of a mushroom))

First, the superfine particles of a mushroom extract of the present invention are described by referring mainly to the production of the superfine particles of a mushroom extract.

[0027]

In the present invention, the type of mushroom is not particularly limited. Further, the site used in extraction is not particularly limited either. Edible mushrooms can be used. Typical examples include, but are not limited to, the followings.

[0028]

The mushroom in the present invention refers to fungi of which fruit bodies has grown, or the fruit bodies thereof.

[0029]

Lentinus edodes

Pleurotus ostreatus

Pholiota nameko

Flammulina velutipes

Tricholoma matsutake

Lyophyllum shimeji

Schizophyllum commune

Crepidotus variabilis

Lyophyllum ulmarium

Grifola umbellata

G. frondosa

Coriolus versicolor

Fomes fomentarius

Volvavella volvacea

Auricularia aurcula-judae

Ganoderma lucidum

G. applanatum

Fomitopsis pinicola

Dictyophora indusiata

Sparassis crispa

Agaricus blazei

Peziza vesiculosa

[0030]

The used site of the mushroom is not particularly limited to special regions such as fruit body, mycelium etc. as described above. The fruit body can be used in order to be able to extract a larger amount of the active ingredients. The components of a raw mushroom are varied depending on the type of mushroom, and for example, a shiitake fruit body is composed of about 90% (by weight) water, about 5% (by

weight) sugar, about 2% (by weight) protein, about 1% (by weight) fiber and about 2% (by weight) other components. Accordingly, the active (efffective) ingredient in the present invention is superfine particles of non-water components extracted with water (hot water etc.).

[0031]

It is not particularly difficult to obtain an extract. For example, a mushroom may be used in extraction with water such as hot water etc. In the case, the extraction step can be easily carried out by subjecting its milled material to the step of extraction with hot water. When hot water is used, a temperature of about 60 to 100°C or so is used. A filtrate obtained by removing insoluble matters through filtration after the extraction step, even whether the filtrate is a suspension containing finely pulverized particles or a solution containing aggregates obtained by further concentrating, cooling and the like the suspension, falls under the scope of the extract in the present invention.

[0032]

The extract in the present invention may be a component dissolved in water in the extraction step with water (hot water etc.) described above, and therefore, a homogeneous extract solution obtained by removing insoluble matters through filtration or the like after the extraction step, and the fine particle component (aggregates) coagulated from the extract solution by concentration, cooling etc. also fall under the scope of this extract.

[0033]

As the extracting solvent, it is possible to use not only water but

also other organic solvents, but the extracting solvent is preferably water alone or a mixed solution of water and a small amount of an organic solvent, and as a matter of course, the extraction with such a water-containing solution also falls under the scope of the water extraction in the present invention. Further, even if an acid, an alkali or an inorganic substance is contained in the extracting solvent or added thereto if necessary in such a range that the amount of a mushroom extract is not adversely affected, there is no problem.

[0034]

By further subjecting the extracted component to a step of superfine pulverization, the superfine particles of a mushroom extract having an immune activating activity or an immune regulating activity can be produced.

[0035]

Hereinafter, the production of the superfine particles of the present invention is described in more detail by reference to preferable examples.

[0036]

In an extract of a mushroom with hot water etc., for example in an extract obtained by extraction, then removing insoluble matters through filtering the hot extract (filtration through Celite etc.) and cooling the filtrate or concentrating and then cooling the filtrate, aggregates having an average particle diameter of 100 μm or more are coagulated. The aggregates are considered to be those formed by aggregation of polysaccharides such as β-glucan or peptidoglycan etc.

in the extract. For example, when a extract (after removing insoluble matters) obtained by extracting from a milled raw shiitake mushroom at 95°C for 3 to 15 hours and filtering the extract through Celite is observed, the filtrate was confirmed to be a suspension having finely pulverized particles dispersed in the extract. By measuring the particle diameter of this particle, it was also confirmed that the particle is an aggregate having a median diameter of about 250 μm, and the components of this particle are β-glucan, peptidoglycan etc.

[0037]

When such an extract (extract containing aggregates each having an average particle diameter of 100 μm or more) is orally ingested or administered, the active (effective) ingredient in the extract is not efficiently absorbed through a mucosa in the intestinal tract and is thus not effectively utilized in the living body. According to the superfine particles of the present invention, on the other hand, the active ingredient in the extract can be efficiently absorbed or incorporated through a mucosa in the intestinal tract to induce or cause immune reaction in lamina propria mucosae.

[0038]

That is, the mushroom extract solution containing aggregates obtained by removing insoluble matters through filtration from a hot extract of a mushroom with water (hot water etc.) and then cooling the filtrate or concentrating and cooling the filtrate is dispersed with an emulsifier etc., particularly an emulsifier capable of forming micelles such as lecithin, to let micelles capture a mushroom extract as well as

to disperse the coagulated aggregates using an emulsifier, whereby superfine particles having an average particle diameter minimized to preferably 10 μm or less, more preferably 1 μm or less, still more preferably 0.01 to 1 μm or so can be produced.

[0039]

For superfine pulverization of the active ingredient or coagulated aggregates in the mushroom extract, a complex between the active ingredient of the mushroom extract dissolved therein and micelle can be formed, the coagulated aggregates can be dispersed with a micelle or emulsifier, or the active ingredient of the mushroom extract dissolved therein can be embedded in liposomes or microcapsules etc., or the coagulated aggregates can be dispersed with an emulsifier and then embedded in liposomes or microcapsules etc.

[0040]

Whether an immune activating action is present or not can be easily confirmed by measuring an antitumor activity, an NK (natural killer) activity, delayed type hypersensitive reaction, an amount of intracellular and extracellular cytokines, or the like.

[0041]

The method of superfine pulverization is not particularly difficult, and superfine pulverization can be effected by using, for example, an emulsifier (machine for emulsification) and a suitable emulsifier (emulsifying agent).

[0042]

When an emulsifier is used, the emulsifier (emulsifying agent) is

not particularly limited insofar as it is an edible emulsifiers. Examples thereof include lecithin, lysolecithin, bile acid etc.

[0043]

When the superfine particles of a mushroom extract of the present invention are measured in the form of a dispersion in water, the superfine particles having an average particle diameter of preferably 10 μm or less, more preferably 1 μm or less, still more preferably 0.01 to 1 μm or so can be used. When used as an immune activator/immune regulator, a solution (emulsified solution) of the superfine particles treated with an emulsifier, particularly a micellar solution or a solution in the liposome form is preferably used for digestion and incorporation, but the superfine particles in a dried state can also be used as an immune activator/immune regulator.

[0044]

In the present invention, the method of measuring (determining) the superfine particles can be carried out by utilizing a method of measuring usual particles, particularly dispersed particles. For example, the superfine particles can be measured by a laser diffraction/scattering particle size distribution measuring method using a particle size distribution meter.

[0045]

(Immune activator/immune regulator)

As described above to some degrees, the superfine particles of a mushroom extract of the present invention can be utilized as the active ingredient of an immune activator/immune regulator (immune

activator/immune regulator of the present invention). A carrier or an excipient (bulking filler) (or a diluent) usable in the pharmaceutical composition or the food and drink (food and/or drink) according to the present invention can also be used. Specifically, the immune activator/immune regulator can be used as the pharmaceutical composition, the food and drink (food and/or drink) (health foods etc.) and the like.

[0046]

Whether an immune activating action or immune regulating action is present or not can be easily confirmed by measuring e.g. an antitumor activity, an NK activity, delayed type hypersensitive reaction, or an amount of intracellular and extracellular cytokines.

[0047]

(Pharmaceutical composition)

The pharmaceutical composition (agent; drug; pharmaceutical preparation) of the present invention is an agent (a pharmaceutical preparation) which comprises the superfine particles of a mushroom extract as described above, preferably the solution treated with an emulsifier (emulsifying agent) (emulsified solution), more preferably the solution containing the micellar component(s) as the active ingredient and which can be used for treatment, amelioration and prevention from enlargement, of diseases accompanying abnormalities in immunity or for prophylaxis etc. of other diseases by activating or regulating immunity, particularly systemic immunity. For example, the pharmaceutical composition can be used for an antitumor agent, an

anti-infective agent, an antiviral agent, an anti-autoimmune disease agent, an anti-diabetes agent and an anti-allergy agent, and the like, and used for treatment or prevention (preservation) of these various diseases.

[0048]

The subject to which this agent (pharmaceutical preparation) is applied is an animal, particularly a human seeking activation or regulation of immunity, particularly systemic immunity.

[0049]

One characteristic of the pharmaceutical preparation of the present invention is that an excellent effect is brought about even by oral administration; and the pharmaceutical preparation is particularly excellent in safety in order to use of an extracted mixture from a mushroom, particularly from an edible mushroom. Accordingly, the form of administration is not particularly limited. Various forms of administration such as oral administration, parenteral administration (subcutaneous administration, intramuscular administration etc.) can be used, and the pharmaceutical preparation can be applied widely and easily to patients seeking an immune activating action and/or an immune regulating action. The pharmaceutical preparation is suitable for safety and oral administration, and can thus be used in the form of health foods, functional foods, health drinks, functional drinks etc. described later, in order to prevent and ameliorate the intended disease.

[0050]

In the present invention, the pharmaceutical preparation can be

mixed or combined with other pharmaceutical component(s) (pharmaceutically active substance(s)), and insofar as a certain pharmaceutical preparation comprises the desired active ingredient in the present invention to exhibit the desired pharmacological activity (immune activating activity or immune regulating activity), such pharmaceutical preparation falls under the scope of the pharmaceutical preparation of the present invention.

[0051]

In addition, the pharmaceutical preparation can further contain a wide variety of pharmacologically acceptable pharmaceutical material(s) (as adjuvant etc.) for pharmaceutical preparation. The pharmaceutical material(s) can be selected suitably depending on the form of the preparation, and examples thereof include excipients, diluents, additives, disintegrating agents, binders, coating agents, lubricants, sliding agents, lubricants (lubricant pharmaceuticals), flavorings, sweeteners, emulsifiers (emulsifying agents), solubilizers etc. Further examples of the pharmaceutical materials include magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, milk protein, gelatin, starch, cellulose and derivatives thereof, animal and vegetable oils, polyethylene glycol, and solvents such as sterilized water and monovalent or polyvalent alcohols, for example glycerol.

[0052]

The pharmaceutical preparation of the present invention can be prepared in various pharmaceutical forms known in the art or to be

developed in the future as described above, for example in administration forms for oral administration, intraperitoneal administration, transdermal administration, inhalation administration etc. To prepare the agent (pharmaceutical preparation) of the present invention in such various pharmaceutical preparation forms, methods known in the art or to developed in the future can be suitably used.

[0053]

The forms of these various pharmaceutical preparations include, for example, suitable solid or liquid pharmaceutical forms such as granules, powders, coated tablets, tablets, (micro)capsules, suppositories, syrups, juices, suspensions, emulsions, dropping agents, injection solutions, preparations prolonging release of the active agent, etc.

[0054]

As a matter of course, the pharmaceutical preparation of the present invention in the pharmaceutical preparation forms illustrated above should contain a pharmaceutically effective amount of the above described component(s).

[0055]

The amount of the pharmaceutical preparation of the present invention administered is selected suitably depending on the type and severeness of the intended disease, the form of the pharmaceutical preparation, etc. For example, the superfine particles of the active ingredient can be administered orally to a patient in a daily dose of preferably 1 mg to 50 g or so, more preferably 10 mg to 10 g or so, still

more preferably 50 mg to 1 g or so expressed in terms of the total net weight based on the dry weight thereof. In the case of severer diseases, the dose can be further increased. With respect to the frequency and intervals of administration, the pharmaceutical preparation of the superfine particles can be administered once every a few days or once every day, but is usually administered for example before, between and/or after meal (or each meal) in 2 to 4 divided portions several times every day. Preferably, the pharmaceutical preparation of the superfine particles is administered before meal. In the case of intravenous administration, the dose may be one tenth to hundredth (1/10 to 1/100) as small as the dose in oral administration.

[0056]

(Food and drink)

Even when the food and drink (food and/or drink) of the present invention are used particularly as health foods or functional foods, the food and drink can be prepared on the basis of the above-described oral preparation by adding component(s) (including extract(s) derived from different mushroom(s)) and additives necessary for health foods or functional foods. As a matter of course, edible or nutrient ingredients etc. used in food and drink can be added if necessary and used. Usually, the superfine particles (in a dried state) can be contained in an amount of preferably 0.01 to 20% or so by weight, more preferably 0.1 to 10% or so by weight, still more preferably 1 to 5% or so by weight.

[0057]

Flavorings or sweeteners usable in food and drink can be used to

form a solution usable in the form of drink or a form in the form of tablets, granules or capsules, or a form in a jelly or ice cream form, or one in a frozen form or the like.

[0058]

The food and drink can be used for prevention not only for healthy persons but also for patients with severe to light various diseases, particularly for patients seeking systemic immunity activation or immunity regulation without limitation to patients with diseases accompanying abnormalities in immune functions.

[0059]

[Embodiments]

Hereinafter, the present invention is described in more detail by reference to Production Examples, Examples and Comparative Examples, but the present invention is not limited to these examples.

[0060]

(Example 1)

(Method of extraction from shiitake mushroom)

About 4 L water was added to per kg (in terms of raw shiitake mushrooms) of fruit bodies of shiitake mushrooms, and the mushrooms were disrupted with a colloid mill (volume of the solution after disruption: about 6 L) and boiled at 95°C for 15 hours under heating under reflux to prevent evaporation of water, and the resulting extract was filtered. The filtrate was concentrated at 60°C under reduced pressure to give about 1 L concentrate. The extract was analyzed for its sugars by a phenol-sulfuric acid method, indicating that the content

of sugars in the extract was a 20 mg/ml concentration (this extract may be referred to hereinafter as "extract of shitake"; or “shitake extract”).

[0061]

(Treatment of the shiitake extract with an emulsifier (emulsifying agent) [conversion into micelles])

Lecithin (SLP-PC70) manufactured by Tsuru Lecithin Kogyo Co., Ltd. was added to deionized water to prepare a solution containing lecithin at the same concentration as that of the whole sugar in the shiitake extract. To the lecithin solution was added the same volume of the above shiitake extract, and the mixture was stirred under vacuum (vacuum pressure: −60 cmHg; number of revolutions (rotation frequency) of an anchor mixer: 50 rpm; number of revolutions of a homomixer: 15,000 rpm) by an Agi homomixer 2M-2 model manufactured by Tokushu Kika Kogyo Co., Ltd., to prepare a preliminary micellar solution. The resulting preliminary micellar solution was subjected to high-pressure emulsification treatment (emulsification pressure 1,500 kgf/cm^2) with a high-pressure emulsifier H11 model in a 2-step handle system, manufactured by Sanwa Kikai Co., Ltd., to prepare a micellar solution of the shiitake extract having a median diameter of about 100 nm (micellar shiitake extract: the product of the present invention). Measurement of the median diameter was carried out by a laser diffraction/scattering particle size distribution measurement method using an LA-910 particle size distribution meter manufactured by Horiba Seisakusho Co., Ltd.

[0062]

(Example 2)

(Example using an S180 subcutaneous inoculation model)

(Test method)

Sarcoma 180 tumor cells maintained by intraperitoneal injection in ICR mice (female, 4-week-old) were collected in the form of ascitic fluid and prepared at a density of 3×10^7 cells/ml with physiological saline. This cell suspension was subcutaneously inoculated in a volume of 0.1 ml/mouse through a 25 G needle into a right groin of ICR mice (female, 4-week-old).

[0063]

On the next day, the mice were grouped (7 mice/group) depending on their weight, and were identified, and then administrations of the extract of shiitake (shiitake extract) and the micellar solution of the extract of shiitake (micellar shiitake extract) were initiated. In this administration, the extract was orally administered (one time/day) 5 times per week, and the administration was conducted 10 times in total. The dose for each administration was as follows: the extract (sample) adjusted to a concentration of 1 mg/ml was given in a dose of 0.2 ml/mouse to a 10 mg (in terms of whole sugar)/kg administration group, and the extract (sample) adjusted to 10 mg/ml was given in a dose of 0.2 ml/mouse to a 100 mg (in terms of whole sugar)/kg administration group.

[0064]

The number in the brackets after "Shiitake extract" and "Micellar shiitake extract", shown in the item "Administration group"

in Tables 1 and 2, is the administration dose of sample (unit: mg/kg). In this example, the particles (the superfine particles) subjected to superfine pulverization treatment with an emulsifier (emulsifying agent) are referred to as the micellar shiitake extract, or micellar solution etc.

[0065]

The tumor size and the body weight were measured once per week. From the tumor size, the tumor weight was calculated according to the following formula.

[0066]

Tumor weight (mg) = tumor minimum diameter(mm)^2×tumor maximum diameter(mm) ÷ 2

[0067]

Further, the host weight was also calculated from the tumor weight and body weight.

[0068]

Host weight (g) = body weight (g) – tumor weight (g)

[0069]

From the tumor weight, the degree of inhibition of tumor growth was calculated.

[0070]

Degree of inhibition of tumor growth (%) = (1 – tumor weight of administration group ÷ tumor weight of non-treatment group) × 100

[0071]

From the degree of inhibition of tumor growth in each week, the pharmaceutical effect on this model was evaluated. The results of the

inhibitory effect of the micellar shiitake extract on the tumor growth are shown in Tables 1 and 2.

[0072]

[Table 1] Tumor weight (g)

Administration group	Day16			
	Average (g)	S.E.	t-test	M test
Non-treatment group	2.493	0.246	-	-
Emulsifier only	2.817	0.401	N.S.	N.S.
Shiitake extract (10)	1.844	0.363	N.S.	N.S.
Shiitake extract (100)	2.712	0.522	N.S.	N.S.
Micellar shiitake extract (10)	1.507	0.163	$p<0.01$	$p<0.01$
Micellar shiitake extract (100)	1.744	0.394	N.S.	$p<0.1$

Day 16: 16th day after inoculation of the tumor

t-test: Student's t-test: t-test of each group as compared with the non-treatment group was conducted.

M test: Mannwhitney-U test: Rank test of each group as compared with the non-treatment group was conducted.

N.S., not significant; $p<0.1$, falsely significant; $p<0.01$, significant.

[0073]

In the group administered the micellar shiitake extract, the tumor growth was inhibited gradually as compared with the non-treatment group during the administration period, and on Day 16 (2nd day after the administration was finished), the tumor growth was inhibited significantly ($p<0.01$) in the group orally administered the micellar shiitake extract in an administration dose of 10 mg/kg.

[0074]

[Table 2] Inhibitory effect on tumor growth

Administration group	Degree of inhibition of tumor growth (%)
	Day16*
Non-treatment group	-
Emulsifier only	-13.0
Shiitake extract (10)	26.0
Shiitake extract (100)	-8.8
Micellar shiitake extract (10)	39.6
Micellar shiitake extract (100)	28.8

*: 16th day after transplant of the tumor

[0075]

As is evident from the results shown above, it was confirmed that the superfine particles of the present invention exhibit the desired pharmaceutical effect as compared with the conventional products.

[0076]

(Example 3)

(Quantification of β-1,3-glucan)

Heparinized whole blood samples were collected from mice, and centrifuged to obtain plasma samples. On the other hand, small intestines were excised from mice, and feces were removed from the lumen. The tissue was washed with physiological saline by mixing with vortex mixer. This procedure was repeated three times, and the wet weight of the tissue was measured. 3ml of physiological saline were added to the tissue, and by homogenating with a disperser or the like, and centrifuging the homogenate, 2.5 ml of supernatant was obtained. The amount of β-1,3-glucan was measured by chromogenic synthetic substrate method with the kit of Seikagaku corporation.

[0077]

(Preparation of histologic samples)

Tumors were extirpated from mice, and small intestines with Peyer patches were removed and then fixed in 10% formalin and embedded in paraffin to prepare paraffin sections which were then deparaffined and stained with hematoxylin/eosin, and they were observed under a stereoscopic microscope.

[0078]

Tissues of small intestines in the normal mice, the untreated group in the tumor bearing mice (non-treatment group), the group administered orally the shiitake extract in doses of 100 mg/kg, and the group administered orally the micellar shiitake extract in doses of 100 mg/kg were observed under a microscope, and as a result, the group administered the micellar shiitake extract only showed accumulation of mononuclear cells (lymphocytes, macrophages) in a lamina propria mucosae in the small intestine. Accordingly, it is considered that immune reaction is induced in the lamina propria mucosae in the intestinal tract by administering the micellar shiitake extract.

[0079]

<Assay method of intracellular cytokines>

The assay method of intracellular cytokines are explained briefly.

[0080]

Heparinized cardiac blood samples were collected from mice of the non-administration group, the group administered the shiitake

extract, and the group administered the micellar shiitake extract, respectively, and added with Brefeldin A (purchased from Sigma Chemical Corporation) at 10 μg/ml. Fluorescence staining of CD-4 molecules on the cell surface was performed with fluorescence-labeled anti-CD-4 monoclonal antibody. The blood samples were treated with an equal volume of red blood cell removing reagent (FACS Lysing Solution (purchase from Becton and Dickinson)) for 10 minutes to lyse the red blood cells. After washing with phosphate buffered saline (PBS) supplemented with 0.5% bovine serum albumin (BSA), the cells were treated with 0.5 ml of FACS permiabilizing solution (purchase from Becton and Dickinson) for 10 minutes, washed with PBS containing 0.5% BSA twice, and treated with optimum concentration of fluorescence-labeled anti-interferon-γ monoclonal antibody (anti-IFN γ mAb) or anti-interleukin-4 monoclonal antibody (anti-IL-4 mAb). After washing with PBS containing 0.5% BSA, the positive cells were analyzed by EPICS/XL flowcytometer (manufactured by Coulter Electronics) with System II software program (manufactured by Coulter Electronics). The ratio of IFNγ positive cells to CD-4 positive cells (Th1 positive rate) and the ratio of IL-4 positive cells to CD-4 positive cells (Th2 positive rate) were calculated to compare Th1/Th2.

[0081]

<Assay method of extracellular cytokines>

The assay method of extracellular cytokines are explained briefly.

[0082]

Heparinized cardiac blood samples were collected from mice of the non-administration group, the group administered the shiitake extract, and the group administered the micellar shiitake extract, respectively, in which the red blood cells were removed by the same method as described above. Peritoneal cells were collected by washing with 5 ml of PBS intraperitoneally injected into mice. The cells were prepared at a density of 5 to 10×10^5 cells/ml with RPMI-1640 medium (manufactured by GIBCO) supplemented with 5% FBS, added with Lipopolysaccharide (LPS:manufactured by Difco) at a concentration of 100 ng/ml, and further cultured for 24 hours to obtain the culture supernatants. Cytokines (IL-12, IL-6 and IL-10) in the supernatants were measured by Enzyme-linked immunoadsorbent (ELISA) with various assay kits (manufactured by Endogen). The production amounts of Th1 cytokine (IL-12) and Th2 cytokines (IL-6 and IL-10) in each group were compared.

[0083]

(Method of evaluating delayed type hypersensitive reaction)

The method of evaluating the delayed type hypersensitive reaction is described.

[0084]

From mice each of the non-administration group, the group administered the shiitake extract, and the group administered the micellar shiitake extract, tumors subcutaneously inoculated were resected together with epidermis under anesthesia with ether, and the incision was adhered with Michel needle. They were subjected to DTH

(Delayed Type Hyper Sensitivity) test for tumor antigen on the 4 to 6 weeks after the resection. That is, 50 μl physiological saline was administered as the control into the right foot pad, while 50 μl tumor antigen solution obtained from sarcoma 180 cells by a 3 M KCl solubilization was administered into the left foot pad, and 24 hours later, the thickness of each of the right foot pad and the left foot pad was measured, and the swelling of the foot was calculated from the following equation, to evaluate the DTH reaction.

[0085]

Swelling of foot (mm) = thickness of left foot pad (mm) – thickness of right food pad (mm)

[0086]

<Assay method of NK activity>

Assay method of NK activity is explained as follows.

[0087]

YAC-1 tumor cells passaged and maintained *in vitro* are prepared in an amount of 2 to 3 times more than necessary. The supernatant is removed by centrifugation at 2,000 rpm for 5 minutes. The cells are suspended in 0.5 ml of DMEM medium (manufactured by GIBCO) supplemented with 5% FBS (Fetal Bovine Serum), mixed with 100 μl of radioactive Sodium chromate ($Na_2{}^{51}CrO_4$), and incubated in CO_2 incubator for 30 to 45 minutes. After washing with DMEM medium, the viable cell number is counted by trypan blue staining.

[0088]

From mice each of the non-administration group, the group

administered the shiitake extract, and the group administered the micellar shiitake extract, spleens are extirpated, sliced with scissors, and meshed to prepare cell suspensions in DMEM medium supplemented with 5% FBS. To 1 ml of the cell suspension, 100 μl of YAC-1 cell suspension are added (Effector cell: Target cell = 10 to 200). After centrifugation at 1000 rpm for 1 minute, the cells are incubated in CO_2 incubator for 4 hours. One drop of 5% of sheep red blood cell (SRBC) is added, and the mixture is centrifuged at 2500 rpm for 5 minutes. The supernatant is collected. The radioactivities of both supernatants and cells are measured by ? counter.

[0089]

The released % of ^{51}Cr calculated by the following equation is regarded as the NK activity.

[0090]

$$\frac{Bs - Bo}{(As + Bs) \times 0.8 - Bo} \times 100$$

wherein Bo: cpm at the supernatant of spontaneous;

As: cpm at the cells of sample;

Bs: cpm at the supernatant of sample

[0091]

[Effect of the Invention]

According to the present invention, there can be provided an immune activator and an immune regulator which can improve an animal, particularly human immunocompetence, particularly a pharmaceutical composition, food and drink (health foods, functional

foods etc.) having such an excellent immune activating action and/or immune regulating action and the like. Further, there can be also provided a novel substance (or a novel composition) usable as an active (effective) ingredient for such excellent products, specifically superfine particles of an extract of a mushroom, preferably a water extract thereof treated with an emulsifier (emulsion), particularly a micellar solution thereof.

[0092]

According to the present invention, there can be further provided a process for producing an extract of a mushroom as an efficacious ingredient by easy production means, particularly the superfine particles of the active ingredient having the immune activating action and/or the immune regulating action, which can produce the above superfine. As a result, a pharmaceutical composition and a food and drink (food and/or drink) (health foods, functional foods etc.) etc. utilizing the active ingredient can be industrially and easily produced. Accordingly, the present invention is industrially and extremely useful.

[Name of Document] ABSTRACT

[Abstract]

[Problems]

The object of the present invention is to provide food and drink (health foods, functional foods etc.) or a pharmaceutical preparation (pharmaceutical composition), which can be prepared by easy preparative means and effectively utilize various components particularly pharmaceutically efficacious components in mushrooms.

[Means for Solution]

By converting an extract of a mushroom such as a shiitake mushroom, into superfine particles, preferably by preparing an aqueous extract thereof treated with an emulsifier, particularly emulsified or micellar solution thereof, the incorporation thereof through mucosa can be improved to demonstrate an immune activating action and/or an immune regulating action. The superfine particles can be used as an immune activator and/or an immune regulator, and thus the invention provides food and drink (health foods, functional foods etc.) and a pharmaceutical composition comprising the superfine particles as an active ingredient, preferably a emulsion obtained by treating the extract thereof with an emulsifier, particularly the micellar solution thereof.

The superfine particles, preferably the emulsion obtained by treating an aqueous mushroom extract with an emulsifier, particularly the emulsified or micellar solution thereof, constitute a novel composition, and the invention also provides this novel composition and a process for easily producing the same.

[Selected Drawing]

None